

Prevalence of extended-spectrum beta-lactamase-producing bacteria in food

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Abstract: Extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* with Cefotaximase–München (CTX-M) enzymes are rapidly increasing worldwide and pose a threat to health care. ESBLs with CTX-M enzymes have been isolated from animals and different food products, but it is unknown if food imported from the Mediterranean area may be a possible reservoir of these bacteria. During 2007–2008, swab samples from food across different retail outlets (mostly food from the Mediterranean countries and Swedish chicken) were collected. *Escherichia coli* strains from Swedish meat and *E. coli* isolates from unspecified food from a Swedish food testing laboratory were also examined. In 349 of the 419 swab samples, growth of *Enterobacteriaceae* was found. In most of the samples, there was also growth of Gram-negative environmental bacteria. Air dry-cured products contained significantly less *Enterobacteriaceae* isolates compared to lettuces; however, none of the examined *Enterobacteriaceae* harbored ESBLs. This study did not support the theory that imported food from the Mediterranean area or Swedish domestic food might constitute an important vehicle for the dissemination of ESBL-producing *Enterobacteriaceae*; however, a spread from food to humans may have occurred after 2008.

Keywords: ESBL, antibiotic resistance, zoonosis, food, *Enterobacteriaceae*

Introduction

The term extended-spectrum β -lactamase (ESBL) was coined by Philippon in 1989.^{1,2} ESBLs are defined as β -lactamases that have the following characteristics: they are transferable; they can hydrolyze penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins); and they can be blocked (in vitro) by β -lactamase inhibitors such as clavulanic acid. The incidence of infections due to resistant *Enterobacteriaceae* has rapidly increased over the last decade and has become a worldwide epidemic.^{3–5} Known risk-factors for colonization or onset of infection with ESBL-producing *Enterobacteriaceae* include: antibiotic use, prolonged and/or recent hospital stay, severe illness, recent surgery, bladder catheterization, use of invasive medical devices, being a resident of long-term care facility, travelling internationally, and being older than 65 years.^{6–10}

The use of antibiotics in Sweden is low when compared with their use across other countries, especially countries in the southern part of Europe. Also, most of these other countries also use more broad-spectrum antibiotics such as cephalosporins and fluoroquinolones in contrast to Sweden where the use of narrow-spectrum penicillins are much more common.^{11,12} In parallel, these countries have a much higher frequency of antibiotic resistant bacteria, such as ESBL-producing *Enterobacteriaceae*. In

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Sweden, the historical prevalence of these bacteria in blood isolates has been low (around 1%). However, since 2004 an increased frequency of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* has been noted,³ and the reason for this could be that the bacteria are being transmitted during travel, in overcrowded hospitals, or as a result of poor hand hygiene.^{13–15}

The epidemiology of ESBLs is quite complex, and the wider geographical area as well as the country in which the ESBLs are present, as well as the hospital, community, and host (in most cases, a single patient or a healthy carrier) where the bacteria can be transmitted are some of the many different factors to consider when assessing their occurrence. Some other factors include examining the specific type of bacteria (*E. coli* is more endemic, and *K. pneumoniae* is more epidemic). In addition, there are numerous reservoirs, including the environment (eg, soil and water), wild animals, farm animals, and pets where these bacteria are more likely to be found. Finally, the presence of these bacteria may occur due to transmission from food and water and via direct or indirect contact (person to person). Therefore it is important to evaluate if imported food may constitute as a reservoir and being a part of the rapid increase and the spreading of ESBLs.^{16–19}

The Cefotaximase–München (CTX-M) enzymes are natural β -lactamases that are produced by *Kluyvera* spp., and they are found in the chromosomes of those bacteria, but have also been transferred to a plasmid that carries these enzymes.²⁰ The CTX-M enzymes can be classified into five major groups, which are designated as CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. Each of these includes several plasmid-mediated enzymes. The specific uropathogenic *E. coli* clone ST131, which has been associated with the presence of ESBL CTX-M-15 and quinolone resistance, has probably contributed to the successful spread of ESBL-expressing bacteria around the world.^{21,22}

There is evidence that microorganisms can be transferred from animals to humans, and ESBL-producing *Enterobacteriaceae* with CTX-M and other enzymes have been isolated from animals and different food products.^{19,23} Since *Enterobacteriaceae* such as *E. coli* are normal inhabitants of the intestinal flora in animals, food products can be contaminated directly in the abattoirs or in manure, which is used to promote growth in lettuce and vegetables.

In the northern hemisphere, a majority of fresh vegetables have to be imported during the winter season from countries with a high prevalence of ESBL-producing *Enterobacteriaceae*. To assess whether imported fresh salad

(particularly from the Mediterranean region), vegetables, fruit, poultry, dry ham, and beef could constitute a possible reservoir of bacteria with ESBL, a study was conducted to see if these bacteria could be found in food. The possibility that imported food could harbor ESBL-producing *Enterobacteriaceae*, and the possibility that this food could offer a potential explanation as to why the rapid increase of ESBLs has occurred in Sweden (or other parts of the world) has not been investigated. In the current study, imported foods and Swedish chicken and beef products were investigated to see if ESBL-producing bacteria could be found in these food sources.

Materials and methods

A total of 419 swab samples from different retail foods were collected during the winter of 2007–2008. Of these food samples, 385 were from imported food and 34 were of domestic origin. The food samples were collected from six different local supermarkets in the Malmö area. A sterile medical gauze pad, one for each specimen, was used to swab all over each sample, and the samples were put in 8 mL of peptone water. If the transport was delayed, the samples were chilled overnight or, if possible, directly sent to the Clinical Microbiology Laboratory in Malmö, Sweden.²⁴ Ninety-nine *E. coli* strains collected from Swedish meat and 94 *E. coli* isolates acquired from unspecified food received from a Swedish food testing laboratory were also investigated. These isolates were identified in accordance with methods described by the Nordic Committee on Food Analysis.²⁵

The specimens and *E. coli* isolates that were collected from food were inoculated on 32 agar plates (a selective medium for Gram-negative rods) where the samples were analyzed qualitatively without pour plating. A standard identification procedure to differentiate between the specimens and to identify *Enterobacteriaceae* was used.²⁶ The specimens were also inoculated on plates with a medium that was selective for cephalosporin resistance (ChromID™ ESBL; BioMerieux SA, Marcy l'Etoile, France). Any growth on these plates was further examined for ESBL production through synergy testing with discs containing ceftazidim and cefotaxim, as well as amoxicillin/clavulanic acid.²⁷ If any ESBL-producing *Enterobacteriaceae* would be found the strain will be characterised to the species level by phenotypic tests carried out according to national guidelines. The 419 swab samples from food were also examined for Gram-negative environmental bacteria such as *Pseudomonas* spp, *Stenotrophomonas* spp, and *Acinetobacter* spp.

Statistical methods included analysis of the contingency table (Fisher's exact test). Analysis was performed using GraphPad software (GraphPad Software, Inc, La Jolla, CA). The prevalence of Gram-negative bacteria was calculated as the percentage of each food specimen or the percentage identified from food samples from different countries.

Results

Of the 385 swab samples collected from imported food, 60 (16%) showed no growth of Gram-negative rods. In 316 of the swab samples there was growth of *Enterobacteriaceae*. In most of the samples there was also growth of both Gram-negative environmental bacteria and *Enterobacteriaceae*, which were not further identified (311/385). None of the *Enterobacteriaceae* harbored any ESBLs. There were no significant differences in the amount of food containing *Enterobacteriaceae* when comparing countries from the Mediterranean region (Table 1). In 33 of the 34 swab samples taken from Swedish chicken, *Enterobacteriaceae* was found to be mixed with Gram-negative environmental bacteria. None of these *Enterobacteriaceae* harbored ESBLs. No ESBLs were found in the 99 *E. coli* isolates collected from the Swedish meat, or in the 94 *E. coli* isolates from the Swedish food testing laboratory.

Air dry-cured products such as ham, sausage, and beef contained significantly less *Enterobacteriaceae* isolates (3/42) than vegetables (142/157), fresh herbs (24/27), and salads (130/134) ($P < 0.0001$). The air dry-cured products also contained significantly less Gram-negative environmental bacteria (4/42) compared to salad (133/134), fresh herbs (27/27), and vegetables (141/147) ($P < 0.0001$) (Table 2).

Discussion

In the present study, no ESBL-producing bacteria were found in the 385 swab samples or in the 193 *E. coli* strains received from the food testing laboratory. ESBL-producing bacteria

were also not detected in the Swedish meat or in the Swedish chicken; however, significantly more *Enterobacteriaceae* were found in lettuces than in air dried, cured products.

This study has some limitations. First, no ESBL-producing *Enterobacteriaceae* were detected. However, a lot of *Enterobacteriaceae* isolates were found, and only a few of the swab samples showed no growth of Gram-negative rods; if there were any ESBLs, they would have been detected. Second, no quantitative method was used, and pre-enrichment of the samples was not done, which is a method that has often been used in other food studies. In the present study, selective isolation methods were used, and the objective was not to quantify the amount of bacteria, but to find *Enterobacteriaceae*. Third, the study was performed in 2007 and 2008; thereafter, the prevalence of ESBL-producing bacteria has increased in Sweden, so the spreading of these bacteria may have started later.

Little is known about whether transfer of ESBL-producing bacteria occurs between food and humans, but it is well known that the endogenous fecal flora of animal origin can spread across the food chain and transiently colonize the human gastrointestinal system. It is also known that resistant *Enterobacteriaceae* (for example, salmonellosis) in food can be transmitted in the community.^{17,28} The earliest finding of ESBL-producing bacteria in an animal was first reported from Japan in 1988, but it is only during the last few years that ESBL-producing bacteria have been of interest in human medicine and have been isolated from animals.^{18,29} Most of these studies have found ESBL-producing bacteria in meat products. For example in a study from the Czech Republic, ESBL-producing *E. coli* isolates were found in 8 (20%) of 40 turkey farms.³⁰ A study from Tunisia showed that 13 out of 79 (16%) food samples from different supermarkets and butcherries harbored ESBL-producing *E. coli*, and reported that the CTX-M-1 group was the most dominant (10/13).³¹ In one of the first and largest studies of ESBL-producing

Table 1 Culture results from various food samples (n = 385) imported from Mediterranean countries and Brazil analyzed qualitatively at the Clinical Microbiology Laboratory in Malmö, Sweden

Bacteria	Countries involved in the study						Total
	France	Italy	Spain	Turkey	Brazil	Miscellaneous*	
No Gram-negative rods	0	34	20	1	5	0	60
<i>Enterobacteriaceae</i>	0	0	2	1	1	1	5
Gram-negative environmental bacteria	1	6	1	0	1	0	9
<i>Enterobacteriaceae</i> and Gram-negative environmental bacteria	21	124	148	9	4	5	311
<i>Enterobacteriaceae</i> with ESBLs	0	0	0	0	0	0	0
Total	22	164	171	11	11	6	385

Note: *Egypt, Israel, unspecified "non-Swedish," and mixed France, Germany, and Spain.

Abbreviation: ESBLs, Extended-spectrum β -lactamase-producing *Enterobacteriaceae*.

Table 2 Culture result from different food specimens

Bacteria	Fish	Fruit	Duck	Fresh herbs	Vegetables	Cured meat	Beef	Salad	Total
No Gram-negative rods	1	2	0	0	14	38	5	1	60
<i>Enterobacteriaceae</i>	1	1	0	0	2	0	1	0	5
Gram-negative environmental bacteria	0	0	0	3	1	1	1	3	9
<i>Enterobacteriaceae</i> and Gram-negative environmental bacteria	0	2	8	24	140	3	4	130	311
<i>Enterobacteriaceae</i> with ESBLs	0	0	0	0	0	0	0	0	0
Total	2	5	8	27	157	42	11	134	385

Notes: Fruit: fig. Duck: French duck breast. Fresh herbs: parsley, dill, chili pepper. Vegetables: bean, chive, Swede, eggplant, summer squash, cucumber, bell peppers, and tomato. Cured meat: prosciutto, bresaola, mortadella, chorizo, and salami.

Abbreviation: ESBLs, Extended-spectrum β -lactamase-producing *Enterobacteriaceae*.

bacteria in food from Spain in 2003, three of the 866 samples collected from cooked food were ESBL-positive; these samples came from two salads and one chicken. Of 131 raw meat samples from the same study, 35 (27%) were ESBL-positive. Twenty-seven (57%) of 47 retail chicken samples were also positive. Seven (58%) of 12 rabbit samples and one (5%) of 20 studied lamb samples were also positive.³²

In a recent study from the Netherlands, Leverstein-van Hall et al found that 94% of a representative sample of chicken meat was contaminated with ESBL-producing *E. coli*, of which 39% belonged to genotypes also found in human samples.¹⁸ Most of the studies have focused on meat products, but they are usually cooked before eaten, which is why any *Enterobacteriaceae* will die during these procedures. It should be noted that this is not the case with fresh salad, fruit, and vegetables. In this study, significantly more *Enterobacteriaceae* were detected in lettuce and vegetables than in meat. Popular foods imported from the Mediterranean area that are eaten raw – such as air-dried ham (prosciutto and jamón serrano), air dried salted beef (bresaola), and sausage (salami, mortadella, and chorizo) – may also be a source of ESBL-producing bacteria, but in this study, they contained significantly less *Enterobacteriaceae* compared to lettuce and vegetables.

Studies have shown that patients who traveled outside Europe (especially to countries in the Middle East and South East Asia) were at high risk of becoming colonized with ESBL-producing *Enterobacteriaceae*.^{8,9} This might be a more important cause of acquiring ESBL-producing bacteria than consuming imported food from the Mediterranean countries.

It is important to note that this study did not include food samples from the Middle East and South East Asia, and any future studies should concentrate on food samples (especially salad) obtained from these countries since the transfer of ESBL-producing bacteria from food to humans probably occurs during travel. In conclusion, the present study did not support the theory that imported food from the Mediterranean area or

that Swedish food may constitute an important vehicle for the dissemination of *Enterobacteriaceae* with ESBL during 2007 and 2008, and if there is a spread of ESBL-producing bacteria in imported and domestic food, it has begun after 2008.

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